Please cancel claims 5, 15 and 32 as set forth above, without prejudice.

REMARKS

By the above amendments, applicants have amended the claims to define the invention more particularly and distinctly so as to overcome the rejections and to patentably define the invention over the prior art.

OBJECTIONS TO PRIORITY AND THE OATH/DECLARATION (Item #3)

The specification claiming priority to application 09/248,388 and provisional applications 60/074,535, 60/110,279, and 60/110,202 was objected to as failing to provide adequate support under 35 U.S.C. 112 as no mention of the instant invention has been made in any of the prior applications.

For CIP applications, the proper priority date depends upon whether "the disclosure of the application relied upon reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter." (Augustine Med., Inc. v. Gaymar Indus., Inc. 181 F.3d 1291, 50 USPQ 2d 1900, 1908 (Fed. Cir. 1999)). Support for this possession can be found explicitly in the description or implicitly such that a person of ordinary skill would understand, at the time the application was filed, that the limitation is required. (Hyatt v. Boone, 146 F.3d 1348, 1353, 47 USPQ2d 1128, 1131 (Fed. Cir. 1998)).

Applicants suggest that explicit limitations to the claims of the present invention exist in the written description of application 09/248,388 (now US 6,365,362) whose benefit is sought. More specifically, the essential elements of the present invention are, in part, described in Example 2. Example 2 describes an internal tumor cell control which is spiked into whole blood to assess the isolation and immunomagnetic enrichment process prior to analysis by microscopy and flow cytometry (US 6,365,362; Col. 22; line 16 to line 21).

In US 6,365,362, Column 24; lines 5 - 25 describes a procedure for using fluorescently labeled SKBR-3 cells added to whole blood. While this procedure is able to detect 10 to 17 pre-labeled tumor cells (US 6,365,362; Col. 24; lines 58 – 60) of the 20 cells added to 5 ml of blood (US 6,365,362; Col. 24; lines 11-12), this does not give the

confidence obtained in redundant labeling. But, there is an implicit reference to the importance of further (redundant) labeling, especially in clinical samples (US 6,365,362; Col. 25; line 0-8). Here, an average recovery of only 67% was needed (US 6,365,362; Col. 24; line 50), so further detection was not necessary. In assays like CellSpotter® and to minimize the risk of false positives when very low (1-2 rare cells in a sample), it would be inherently apparent that further labeling is necessary to ensure detection, possibly labeling alternate antigenic sites.

The necessity of having cellular components and antigenic moieties (i.e. determinants) in common with rare cells is illustrated in Example 8 (US 6,365,362; Col. 30; line 60 to Col. 31; line 29). In spite of a lack of detection by needle biopsy, malignant cancer cells have been shown to be present after gentle massage of an enlarged prostate. Following isolation of these cancer cells from the circulation, they are used to assess a variety of malignant cell characteristics. Accordingly, these characteristics are common determinants that provide a unique feature for sensitive detection.

Finally, there would be an implied requirement for the use of stable cells as controls. As described in the test kits, positive cells are included in the kits' components and used in screening of patients for circulating rare cells (US 6,365,362; Col. 31; line 35-41). More specifically the test kit of Example 9 would necessarily have to contain positive stable cells in order to control for various reagents and assays in facilitating diagnosis over multiple patients and across multiple clinical sites, as recognized in the present application (page 2, para 0019) and previously supported (US 6,365,362; Col. 31; lines 60-65).

Further, there is an implicit suggestion for the use of a fixed, stable control cell from Example 8 (US 6,365,362; Col. 31; line 25-28), "isolated cells can be readily cultured and/or cloned" where the use of these stable cell lines are for a variety of different purposes, including controls. It would be reasonable that other forms of stable cells (i.e. permeabilized then fixed) would function as control, much like the stable cell lines described.

The applicants respectfully suggest that both explicit limitations and implications, as understood by a person of ordinary skill, exist in the specifications of US 6,365,362 and should be entitled to the earlier priority. As this issue is related to objections in the

oath/declaration, applicants request the opportunity to rectify the oath/declaration in the manner suggested by the examiner should the earlier priority not be reinstated as requested.

REJECTION OF CLAIMS 5, 15 AND 32 UNDER 35 U.S.C. 112, 2ND (Item #5)

Applicants agree with the examiners rejection. Accordingly, claims incorporating trade names have been canceled.

REJECTION OF CLAIMS 1-23, 27-42, 46-50 AND 54 UNDER 35 U.S.C. 112, 2ND (Item #6)

Redundant labeling as recited in claims 1, 7, 20, 28 and 37 has been rejected because of the lack of clarity in what encompasses redundant labeling. These claims have been rewritten to include "at least two distinct fluorescent labels possessing overlapping emission spectra". Selecting a single detection gate within the overlapping spectra from distinct labels provides the redundant labeling needed to ensure internal control cell detection.

Redundant labeling, as described, can be by either concurrent or sequential addition of a second pre-label. For example, the octadecyl indocarbocyanines (DilC18) and the octadecyl oxacarbocyanines (DiOC18) have an emission spectra of 530 to 700 nm and 450 to 650 nm, respectively (see "Handbook of Fluorescent Probes and Research Chemicals" Haugland R.P. 5th ed., 260; 1994). These are two distinct fluorescent labels possess overlapping emission spectra. Selecting a single detection gate within the overlapping spectra provides the redundant labeling to ensure internal control cell detection (page 9, para 0093). Support for this change is exemplified with the fluorescent labeled HER81 antibody in the specification (page 10, para 0094).

Accordingly, applicants suggest that the term "distinct" clarifies redundant labeling and request reconsideration of the rejection in light of the above stated change.

REJECTION OF CLAIMS 10-21, 28-41, 46-50 AND 54 UNDER 35 U.S.C. 112, 2ND (Item #11)

The phrase "previously permeabilized" was rejected because it was unclear how a "previous" permeabilization affects the immediate properties of a stabilized cell.

Applicants agree with the examiner's interpretation and the claims have been amended to recite a permeabilized, stabilized cell.

REJECTION OF CLAIMS 22, 23, 41, 42, 49 AND 50 UNDER 35 U.S.C. 112, 1ST (Item #12)

The use of a detectably labeled surface determinant which is an estrogen determinant or an androgen determinant was rejected for not reasonably providing the enablement needed for surface detection. Applicants suggest that there is ample support in the literature to support the existence of estrogen and androgen receptors outside the nucleus and on the plasma membrane.

1- "Cellular functions of plasma membrane estrogen receptors" Levin, E.R. Steroids 2002; 67; 471-475.

2- "Plasma membrane estrogen receptors signal to anti-apoptosis in breast cancer" Razandi M, Pedram A, Levin E.R. Mol Endocrinol 2000; 14; 1434-1447.

3- "An estrogen membrane receptor participates in estradiol actions for the prevention of amhyloid-beta peptidel-40-induced toxicity in septal-derived cholinergic SN56 cells" Marin R., Guerra B., Morales A., Diaz M., Alonso R. J Neurochem 2003; 85; 1180-1189.

4- "Regulation of the membrane estrogen receptor-alpha: role of cell density, serum, cell passage number, and estradiol" Campbell C.H., Bulayeva N, Brown D.B., Gametchu B., Watson C.S. FASEB J 2002; 16(14); 1917-1927.

5- "Membrane-associated binding sites for estrogen contribute to growth regulation of human breast cancer cells" Marquez D.C., Pietras R.J. Oncogene 2001; 20(39); 5420-5430.

REJECTION OF CLAIMS 1, 2, 4, 5, 6, 10, 12, 14, 15, 28, 29, 31, 32, AND 33 UNDER 35 U.S.C. 103(A) (Item #13)

Claims 1-2-4-5-6-10-12-14-15-28-29-31-32-33 were rejected as unpatentable over the combination of Davis, Leif et al, Terstappen et al, Maples et al and any of the abstracts of Labalette-Houache et al or Decaestecker et al or Yang et al, the combination being motivated by Gross et al and Terstappen et al.

Applicants submit that Gross et al does not take into consideration the targets for internal controls, the level of labeling efficiency needed, and the means for multiple fluorochrome labeling. Absent these factors, when taken together, no motivation exists to combine the prior art.

The function of control cells in the present invention are very different than any other control cells, previously described in the art, as they control for several very different variables. These variables include (1) the cells control for magnetic labeling reagent, i.e. the integrity of the ferrofluids (2) the cells control for the integrity of the magnetic separation devices used in the process (3) the cells control for the integrity of labeling agents used following the enrichment process and (4) the control cells give the operator a good indication of the efficiency of the enrichment process and/or the detection-analysis portion of the assay (page 2, para 0022).

The control cells of the present application are labeled with high efficiency in order to be able to introduce internal control cells which are morphologically identical to the target cells. The efficiency of labeling goes from 99.99% for single labeling (with 1 in 1000 unlabeled or dimly labeled) to greater than 99.9999% efficiency for redundant labeling (1 in 10^6 cells unlabeled or dimly labeled; page 10, para 0094). Gross et al. provides a manner to only detect a low frequency of target cells, thus there would not be any suggestion for developing control cells with a high efficiency of labeling.

In fact, Gross et al. uses a covalent method for labeling BT-20 cells with 7-amino-4-chloromethylcoumarin which has a low efficiency of labeling (i.e. labeling at 4°C prior to paraformaldehyde fixation; see Gross et al., page 538, col 1, para 4). The use of this low efficiency labeling suggests that Gross et al. could not provide the motivation necessary to combine the prior art with an expectation of developing the internal control cells of the present invention.

The morphologic characteristics of the control cells in Davis were preserved through freeze-drying and were subsequently labeled after re-hydrating before their use. Neither Gross et al. nor Terstappen et al. provides the motivation to combine both the stability of the antigen-antibody complexes in leukocytes after cross-linking (Leif et al.) and the suggested improvement of differentially labeled control cells over fluorescent beads (Maples et al.) with the teachings from Davis.

One reason for this is that there is no suggestion in Gross et al. to utilize multiple fluorescent entities at one or more cell specific sites (i.e. multiple fluorochromes linked to one or more antibodies for recognition at antigenic sites) in a way that would ensure detecting cell labels through a single spectral window or gate. Gross et al. relied on using multiple antibodies conjugated to a single fluorescent moiety (PerCP) to detect cytokeratins 5, 6, 8 and 18 (Gross et al., page 537, col 2, para 3). Gross et al. was not concerned with ensuring the high level of confidence for individual cell recognition as in the redundant labeling of the present invention. Thus because a major factor suggesting the development of an internal control cell is not present (i.e. single gate detection of distinct fluorochromes), there would be no motivation to pre-label, permeabilize and then fix cells for use as an internal control.

Applicants submit that it would not be obvious to pre-label the control cells with membrane soluble dyes prior to long term storage as it was found that an unexpected characteristic enables the cells to be permeabilized with mild detergents, fixed and stored for extended periods. The cells still retain membrane label through the process as well as the ability to have its internal components available for subsequent labeling at any time up to six months.

Because these cells are extremely fragile, permeabilization reagents will have a significant effect on membrane soluble dyes, causing them to leach out of the cell and change morphologic characteristics (page 9, para 0093). So it is surprising that the control cells of the present invention can be pre-labeled with membrane soluble dyes, permeabilized with mild detergents, fixed and stored, yet retain membrane label throughout the process as well as the ability to have its internal components available for subsequent labeling at any time up to six months.

In rare cell detection assays, most investigators are reluctant to spike control cells into an unknown sample that has the same morphologic and antigenic properties as the cells being detected. Unexpectantly as shown in Example 3 (page 11, Table 1A), applicants have been able to produce control cells of the present invention to overcome these issues, retaining its pre-labeling profile, so the use of internal control cells in a rare cell detection assay will have the confidence that less than 1/10,000 cells might be unlabelled (a critical factor when controlling for the presence of endogenous tumor cells). Thus in spite of the unwillingness of other investigators to add stabilized tumor cells as a control to a rare cell

detection sample, the present invention provides internal tumor control cells that can be safely added to a rare cell detection sample.

Applicants submit that the internal control cells of the present invention fill the unmet need of having a consistent control to safely and consistently assess the errors in multiple rare cell detection assays, run over an extended period of time.

External controls have been used for conventional low sensitivity flow or imaging assays of cells to detect systematic errors, but without the ability to detect random errors that cannot be detected with external controls alone. Unfortunately, external controls are not satisfactory when there is a need to address issues such as operator error, or with a standard that will act as a safe control from assay to assay, machine to machine, and laboratory to laboratory (page 2, para 0021). Thus, a preferred control must be consistently spiked into an unknown sample (internal control), and confidently recover the same controls for systematic comparisons, especially for rare cell analysis (page 4, para 0031).

In general, the concept of singly pre-labeled internal control cells appears intuitively risky, since it involves adding a differentially stained target cells that may be misclassified as target cells. To minimize the risk, the idea of redundantly (dual) pre-labeling of internal control cells prior to the addition of the actual specimens in rare cell analysis was developed. Despite the fact that detection of both random and systematic errors would appear desirable in highly sensitive assays for rare target cells (e.g. circulating cancer cells in blood), no combination of the prior art suggests developing an internal positive control cell with the attributes of the present invention. For example, providing a means to control sensitive detection assays whereby 0 cells may be safely discriminated from the presence of 1 to 100 circulating tumor cells in a patient.

Variable cell loss between laboratories and the difficulty in standardizing assays between laboratories is a constant concern among diagnostic and research laboratories (page 2, para19). Thus while Davis describes a fixed control cell that is used in whole blood analysis after density centrifugation (Davis, col 3, lines 49-58 and col 4, lines 1-6), Leif incubates whole blood with a monoclonal antibody to label subclasses of leukocytes (the addition of a lysing reagent to the cell suspension further decreases the sensitivity of the method by dilution (Leif et al., col 3, lines 43-58) and Maples et al. describe a method

for calibrating flowcytometers with antigenically preserved cells allowing for reproducibility over time (Maples et al., page 9, line 31-35), the combination would not address the unmet need to develop the internal control cells of the present invention in rare cell analysis. For example, the detection level obtained by immunomagnetic enrichment followed by image analysis in rare cell detection and the need to prevent misclassification or false positive diagnosis as an internal control is not considered by any of the prior art (page 2, para 0022).

In further support to the above arguments, applicants refer to the included affidavit, submitted by inventor Herman Rutner and associated supportive Figures A, B, and C [*In re* Klosak, 455 F.2d 1077, 173 USPQ 14 (C.C.P.A. 1972): *evidence of unexpected results must be provided with support for the assertion*].

In other words looking at the subject matter as a whole, there is no motivation to develop internal control cells that could be used in a cell analysis system having this level of sensitivity, especially with respect to image analysis of rare tumor cells in patient diagnosis. In addition, the unexpected results that allow long term use of these control cells fulfills an unmet need to provide a safe, consistent control in assessing multiple types of errors.

REJECTION OF CLAIMS 1-6, 10, 12-15 AND 28-33 UNDER 35 U.S.C. 103(A) (Item #14)

Claims 1-6, 10, 12-15 and 28-33 were rejected as unpatentable over Davis and Leif et al and Maples et al and Terstappen et al and Gross et al and any of the abstracts of Labalette-Houache et al or Decaestecker et al or Yang et al as applied to claims 1-2-4-5-6-10-12-14-15-28-29-31-32-33 above and further in view of Gibson et al and Waggoner et al and Haugland.

Applicants respectfully refer to the reasons set forth in Item #13 above associated with the lack of motivation to combine and unexpected results behind the embodiments of claims 1, 2, 4, 5, 6, 10, 12, 14, 15, 28, 29, 31, 32 and 33.

Further, applicants submit that, because Gibson et al is not aware of the necessary traits in the control cells of the present invention, Gibson et al can not provide the necessary motivation to combine the prior art.

Gibson et al pre-labeled with Hoechst 33342, washed, and then analyzed by fluorescent activated cell sorter (FACS) analysis and fluorescence microscopy. Gibson's use of a glycolipid stain does not address redundant labeling in the context of the present invention whereby membrane labeling would be in conjunction with another fluorochrome (page 9, para0093). Consideration that the dye must possess the excitation/emission spectra needed for redundant labeling and detection through a single fluorescent window yet not interfering with detection of target cells would be a motivating factor in using a glycolipid stain. The dye must also efficiently and uniformly stain cells, substantially irreversibly bind to the cell membrane, have minimal leakage during storage, and are optically stable. These characteristics provide a significant improvement in the risk of introducing unlabeled or dimly labeled control cells to a sample. Accordingly, these traits, if known, would provide the motivation necessary to combine the above references in the context of a glycolipid membrane dye.

REJECTION OF CLAIMS 1-10 AND 12-16 AND 18-21, 23 AND 35-40 UNDER 35 U.S.C. 103(A) (Item #15)

Claims 1-10 and 12-16 and 18-21, 23 and 35-40 were rejected as unpatentable over Davis and Leif et al and Maples et al and Terstappen et al and Gross et al and any of the abstracts of Labalette-Houache et al or Decaestecker et al or Yang et al and Gibson et al and Waggoner et al and Haugland as applied to claims 1-6, 10, 12-15 and 28-33 above and further in view of Racila et al and Terstappen et al (2000) and Xing et al.

Applicants respectfully refer to the reasons set forth in Item #14 and #13 above associated with the lack of motivation to combine and unexpected results behind the embodiments of claims 1-6, 10, 12-15 and 28-33.

Further, applicants submit that the teachings of Racila et al and Terstappen et al (2000) do not consider the unexpected results associated with the internal control cells of the present invention as set forth in Item #13 above.

Neither Racila et al nor Terstappen et al appreciates the ability to store prepared control cells for an extended period of time while maintaining their morphologic and

antigenic profiles. This unexpected result allows for a consistent internal control in assessing rare cell assays.

Finally, applicants respectfully refer to the reasons set forth in Item #3 above associated with objections to priority and Terstappen et al (2000) as prior art.

Applicants' claim for priority has the following dates; 09/248,388 filed Feb. 12, 1999, 60/074,535 filed Feb. 12, 1998, 60/110,202 filed Nov. 30, 1998, 60/110,279 filed Nov. 30, 1998.

REJECTION OF CLAIMS 1-21, 23, 28-40 AND 42 UNDER 35 U.S.C. 103(A) (Item #16)

Claims 1-21, 23, 28-40 and 42 were rejected as unpatentable over Davis and Leif et al and Maples et al and Terstappen et al and Gross et al and any of the abstracts of Labalette-Houache et al or Decaestecker et al or Yang et al Gibson et al and Waggoner et al and Haugland and Racila et al and Terstapen et al and Xing et al as applied to claims 1-10, 12-16, 18-21, 23, 28-33 and 35-40, 42 above and further in view of Young et al.

Applicants respectfully refer to the reasons set forth in Item #15, #14, and #13 above associated with the reasons behind the lack of motivation to combine the prior art and unexpected results embodied in claims 1-10 and 12-16 and 18-21, 23 and 35-40.

Further, applicants submit that Young et al does not consider required knowledge of the characteristics of the buoyant density medium needed to combine the prior art, along with the unexpected results associated with the internal control cells of the present invention and, therefore, does not possess the necessary motivation needed to combine.

Combining Young et al. with the above mentioned art would not provide the motivation required to develop the buoyant density medium claimed in the present invention. Young et al. relates to a suspension media for use in blood counting instruments (Young et al., col 6, lines 30-41). The importance of maintaining homogeneity of fixed, permeabilized stable control cells as described in the present invention is not appreciated by Young. The present invention requires reproducible pipetting from a stock solution that does not require re-mixing and in a medium capable of storing control cells for extended periods without any alteration in the morphologic and antigenic properties of the cells (page 15, para 0134).

In addition, the use of the cells as a control in rare cell detection assays would require a storage medium that would minimize or eliminate erroneous control cell concentrations. These would include, for example, a storage medium whereby the control cells are not incorporated into any foam on the surface of the storage medium, and consequently cause a small change in cell number. Also, storage can not cause the cells to clump, cross-link or stick to the vial. A small change in cell number by any of these would significantly alter error analysis in systems designed to detect only a few rare cells. Young et al does not appreciate these characteristics in describing a suspension medium (Young et al., col 14, line 61 to col 15, line 8) and, therefore, could not be motivated to combine the prior art.

REJECTION OF CLAIMS 1-23, 28-42 UNDER 35 U.S.C. 103(A) (Item #17)

Claims 1-21, 23, 28-40 and 42 were rejected as unpatentable over Davis and Leif et al and Maples et al and Terstappen et al and Gross et al and any of the abstracts of Labalette-Houache et al or Decaestecker et al or Yang et al and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstapen et al and Xing et al and Young et al. as applied to claims 1-21, 23, 28-40 and 42 above and further in view of Rao et al.

Applicants respectfully refer to the reasons set forth in Item #16, #15, #14, and #13 above associated with the reasons behind the lack of motivation to combine the prior art and unexpected results embodied in claims 1-21, 23, 28-40 and 42.

Further, applicants submit that Rao et al does not have the necessary knowledge to motivate the combination of the prior art. More specifically, Rao et al would not appreciate that the recovery of control cells, independent of antigen density, could accurately reflect the recovery of circulating tumor cells and so would not realize the ability to substitute MCF-7 cells for fixed SKBR3 cells.

In breast cancer cells from patients, the EpCAM antigen density varies over 3 log units. Despite this variability, a reproducible percentage of tumor cells is always captured, using immunomagnetic separation techniques (page 12, para 0113). Rao et al did not address this variability. In fact, Rao et al arbitrarily decided to define tumors that

were positive for estrogen receptors as cell populations having at least a 10% labeled population (Rao et al., page 2904, para 1). Without realizing the fluctuation in antigen density, Rao et al could not have substituted MCF-7 for EpCAM. In fact, there would be no motivation to combine the prior art as variations in MCF-7 antigen density could fluctuate over 3 log units. Rao et al would not appreciate this fact in substituting as a suitable control.

Further without the benefit of hind site, Rao et al would not be able to use the MCF-7 cells as a control cell with reproducible recovery. Although the differences in antigen density would not affect recovery following immunomagnetic separation, the ability to substitute MCF-7 cells for fixed SKBR3 cells could only occur if the cells are used as a control in conjunction with the magnetic enrichment of the present invention.

REJECTION OF CLAIMS 1-23, 28-42 AND 47-50 UNDER 35 U.S.C. 103(A) (Item #18)

Claims 1-23, 28-42 and 47-50 were rejected as unpatentable over Davis and Leif et al and Maples et al and Terstappen et al and Gross et al and any of the abstracts of Labalette-Houache et al or Decaestecker et al or Yang et al and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstapen et al and Xing et al and Young et al. and Rao et al. as applied to claims 1-23, 28-42 above and further in view of Terstappen et al (WO 99/41613)

Applicants respectfully refer to the reasons set forth in Item #17, #16, #15, #14, and #13 above associated with the reasons behind the lack of motivation to combine the prior art and unexpected results embodied in claims 1-23, 28-42 and 47-50.

Further, applicants respectfully refer to the reasons set forth in Item #3 above associated with objections to priority and Terstappen et al (WO 99/41613) as prior art.

Applicants' claim for priority has the following dates; 09/248,388 filed Feb. 12, 1999, 60/074,535 filed Feb. 12, 1998, 60/110,202 filed Nov. 30, 1998, 60/110,279 filed Nov. 30, 1998.

REJECTION OF CLAIMS 1-23, 27-42 AND 46-50 AND 54 UNDER 35 U.S.C. 103(A) (Item #19)

Claims 1-23, 27-42 and 46-50 and 54 were rejected as unpatentable over Davis and Leif et al and Maples et al and Terstappen et al and Gross et al and any of the abstracts of Labalette-Houache et al or Decaestecker et al or Yang et al and Gibson et al and Waggoner et al and Haugland and Racila et al and Terstapen et al and Xing et al and Young et al. and Rao et al. and Terstappen et al (WO 99/41613) as applied to claims 1-23, 28-42 and 47-50 above and further in view of Shih (1999), Shih et al (1994) and the abstract of Silverstein et al.

Applicants respectfully refer to the reasons set forth in Item #18, #17, #16, #15, #14, and #13 above associated with the reasons behind the lack of motivation to combine the prior art and unexpected results embodied in claims 1-23, 28-42 and 47-50.

Finally, applicants respectfully refer to the reasons set forth in Item #3 above associated with objections to priority and Shih (1999) as prior art.

Applicants' claim for priority has the following dates; 09/248,388 filed Feb. 12, 1999, 60/074,535 filed Feb. 12, 1998, 60/110,202 filed Nov. 30, 1998, 60/110,279 filed Nov. 30, 1998.

In view of the amendments and foregoing discussion and arguments, it is respectfully urged that the rejections set forth in the February 27, 2003 Official Action should be withdrawn and that this application be passed to issue. In the event the examiner has any comments or questions, the examiner is invited to telephone or e-mail applicants' undersigned representative at the number below.

Respectfully submitted,

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CLEAN VERSION OF AMENDMENTS UNDER 37 CFR 1.121(C)(1)(i)

In the claims:

1. (Amended) A process for producing a stabilized cell for use as an internal control in methods for isolating and identifying rare cells, said stabilized control cell having determinants in common with said rare cells, said process comprising:

a) redundantly labeling said control cell with at least two distinct fluorescent labels having the same spectral properties;

b) permeabilizing said control cell;

c) contacting said labeled cells with a cell fixative said fixative effecting stabilization of both cellular structure and antigenic moieties present on said control cells;

d) subsequently removing the excess fixative to promote long-term storage of said control cells, said control cells being physically and biologically stable for at least six months.

5. (Cancel)

7. (Amended) A process for producing a stabilized cell for use as an internal control in methods for isolating and identifying rare cells, said stabilized control cell having determinants in common with said rare cells, said process comprising:

a) redundantly membrane labeling said control cell with at least two distinct fluorescent labels having the same spectral properties;

b) permeabilizing said control cell;

c) contacting said labeled cells with a cell fixative said fixative effecting stabilization of both cellular structure and antigenic moieties present on said control cells;

d) subsequently removing the excess fixative to promote said long-term storage of control cells, said control cells being physically and biologically stable for at least

six months, wherein said control cell expresses epithelial cell adhesion molecule (EpCam) on its surface and also expresses cytokeratin intracellularly.

10. (Amended) A stabilized cell, permeabilized, for use as an internal control in methods for isolating and identifying rare cells, said control cell having determinants in common with said rare cells, wherein said control cell is labeled redundantly with at least two distinct fluorescent labels having the same spectral properties, and cellular components and antigenic moieties of said control cell have been stabilized for at least six months by exposure to fixative.

15. (Cancel)

16. (Amended) A stabilized cell, permeabilized, for use as an internal control in methods for isolating and identifying rare cells, said stabilized control cell having determinants in common with said rare cells, and comprising a detectably labeled membrane, said cells further comprising stabilized cellular components and antigenic moieties, said stabilization being effected by exposure to a fixative, wherein said control cell is a tumor cell expressing EpCam on its surface and cytokeratin intracellularly.

20. (Amended) The control cell of 16, wherein said membrane is redundantly labeled with at least two distinct fluorescent labels having the same spectral properties.

28. (Amended) A stabilized cell, permeabilized, for use as an internal control in methods for isolating and identifying rare cells, said stabilized control cell having determinants in common with said rare cells, and comprising a redundantly labeled membrane, said membrane being labeled with at least two distinct fluorescent labels having the same spectral properties, said cells further comprising stabilized cellular components and antigenic moieties, said stabilization being effected by exposure to a fixative, wherein said control cell is selected from the group consisting of tumor cells, bacterially infected cells, virally infected cells, myocardial cells, and endothelial cells in circulation, and fetal cells in maternal circulation.

32. (Cancel)

35. (Amended) An improved method for detecting and enumerating rare cells in a mixed cell population, the presence of said rare cells in said population being indicative of severity of a disease state, comprising:

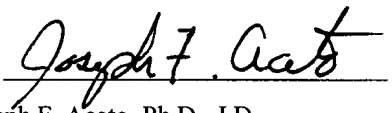
a) obtaining a blood sample from a test subject, said sample comprising a mixed cell population suspected of containing said rare cells;
b) preparing an immunomagnetic sample wherein said blood sample is mixed with magnetic particles coupled to a ligand which reacts specifically with a determinant of the rare cells, to the substantial exclusion of other sample components;
c) contacting said immunomagnetic sample with at least one reagent which labels a determinant of said rare cells; and
d) analyzing said labeled rare cells to determine the presence and number of any rare cells in said immunomagnetic sample, the greater the number of rare cells present in said sample, the greater the severity of said disease state, wherein the improvement comprises the addition of a stabilized cell, permeabilized, for use as an internal control cell in said method, said control cell having determinants in common with said rare cells and wherein said membrane of said control cell is detectably labeled and cellular components and antigenic moieties of said control cell have been stabilized for at least six months by exposure to fixative.

37. (Amended) The method as claimed in claim 35, wherein said membrane is redundantly labeled with at least two distinct fluorescent labels having the same spectral properties.

47. (Amended) An improved kit for screening a patient sample for the presence of circulating tumor cells, comprising:

a) coated magnetic nanoparticles comprising a magnetic core material, a protein based coating material, and anti-EpCAM coupled, directly or indirectly, to said base coating material;
b) at least one antibody having binding specificity for a cancer cell determinant;
c) cell specific dye for excluding sample components other than said tumor cells from analysis wherein the improvement comprises the addition of a container comprising stabilized cells, permeabilized, for use as an internal control, said stabilized control cells having determinants in common with said tumor cells, wherein said membrane of said control cell is detectably labeled, and cellular components and antigenic moieties of said control cells have been stabilized for at least six months by exposure to fixative, said stabilized control cells being suspended in a buoyant density medium.

Respectfully submitted,

By 

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